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Set 6

1-(6-{{3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl}amino}-6-oxohexyl)-
2-[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-
5 ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound IX); and
1-(6-{{2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl}amino}-6-oxohexyl)-
3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-
2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound X).

10 8. A method for labelling a mixture of proteins in a sample wherein
each of said proteins contains one or more cysteine residues, said method
comprising:

- i) adding to an aqueous liquid containing said sample a fluorescent
dye wherein said dye contains a target bonding group that is covalently
15 reactive with said proteins; and
- ii) reacting said dye with said proteins so that said dye labels said
proteins;
characterised in that all available cysteine residues in said proteins are
labelled with said dye.

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9. A method according to claim 8 wherein said fluorescent dye is a
cyanine dye.

10. A method according to claim 9 wherein said cyanine dye contains a
25 sulphonic acid or sulphonate group.

11. A method according to any of claims 8 to 10 wherein said target
bonding group is selected from a maleimido group and an iodoacetamido
group.

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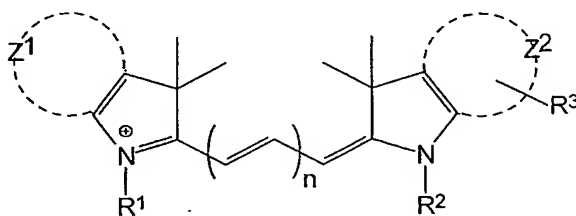
12. A method according to claim 8 further comprising prior to step i), the step of treating the protein with a reductant.

13. A method according to claim 8 wherein said dye is used in a range of 5 to 200nmol of dye per 50µg of protein.

14. A method according to claim 8 wherein said labelling is performed at a pH in the range from 6.0 to 9.0.

15. A method for labelling one or more proteins in a sample, the method comprising:

i) adding to a liquid sample containing said one or more proteins a fluorescent dye of formula (I):

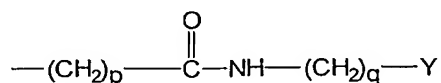


(I)

wherein n is different for each said dye and is 1, 2, or 3;

Z¹ and Z² independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups R¹ and R² is the group:



where Y is a target bonding group;

remaining group R¹ or R² is selected from -(CH₂)₄-W or -(CH₂)_r-H;

group R³ is hydrogen, except when either R¹ or R² is -(CH₂)_r-H, in which case R³ is W;

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W is selected from sulphonic acid and sulphonate;

p is an integer from 3 to 6;

q is selected to be 2 or 3; and

r is an integer from 1 to 5;

5 and their salts;

characterised in that when n of two of said dyes differs by + 1, one of p,

q and r of said two dyes differs by -1; and

ii) incubating said dye with said sample under conditions suitable for
labelling said one or more proteins.

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16. A method according to claim 15 wherein each of Z^1 and Z^2
represents the carbon atoms necessary to complete a phenyl ring system.

17. A method according to claim 15 or claim 16 wherein:

15 n is selected to be 1 or 2;

p is selected to be 4 or 5;

q is selected to be 2 or 3; and

r is selected to be 1, 2 or 3.

20 18. A method according to any of claims 15 to 17 wherein said target
bonding group Y is selected from a maleimido group and an
iodoacetamido group.

25 19. A kit comprising a matched set of fluorescent dyes comprising at
least two different fluorescent dyes having the formula (I):

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